Screening of Blood Parasites in Australian Wild Deer

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Abstract

Wild animals are natural reservoir hosts for a variety of pathogens, and such is the case for deer (family Cervidae). Deer were introduced to Australia 150 years ago for farming and game, but wild deer populations have expanded considerably in recent years, posing increasing threats to biodiversity, agriculture and public health. There are few data currently available on pathogens that Australian wild deer carry or whether these organisms pose biosecurity threats to humans, wildlife, livestock or other domestic animals. To address this knowledge gap, we tested for the presence of seven parasitic genera in 243 blood samples collected from four wild deer species in eastern Australia. Blood samples were tested by PCR for the presence of Plasmodium, Trypanosoma, Babesia, Theileria, Toxoplasma, Sarcocystis and Neospora DNA. No amplification was obtained for either the 18S rRNA (or the cytochrome b gene in the case of Plasmodium) of the seven selected parasitic genera, suggesting that wild deer in eastern Australia currently pose little risk as vectors of these parasites to livestock and humans. This survey represents the first molecular study of its type in Australian deer and provides important baseline information about the health status of these animals.

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Summary

Wild animals are natural reservoir hosts for a variety of pathogens, and such is the case for deer (family Cervidae). Deer were introduced to Australia 150 years ago for farming and game, but wild deer populations have expanded considerably in recent years, posing increasing threats to biodiversity, agriculture and public health. There are few data currently available on pathogens that Australian wild deer carry or whether these organisms pose biosecurity threats to humans, wildlife, livestock or other domestic animals. To address this knowledge gap, we tested for the presence of seven parasitic genera in 243 blood samples collected from four wild deer species in eastern Australia. Blood samples were tested by PCR for the presence of *Plasmodium* ,*Trypanosoma* , *Babesia* , *Theileria* , *Toxoplasma* ,*Sarcocystis* and *Neospora* DNA. No amplification was obtained for either the 18S rRNA (or the cytochrome b gene in the case of *Plasmodium*) of the seven selected parasitic genera, suggesting that wild deer in eastern Australia currently pose little risk as vectors of these parasites to livestock and humans. This survey represents the first molecular study of its type in Australian deer and provides important baseline information about the health status of these animals.

Keywords

Wildlife disease, surveillance, wild deer, parasites, Australia, 18S rRNA gene

Introduction

The frequency of emerging and re-emerging infectious diseases outbreaks in wildlife reservoirs has increased during recent decades (Gortazar, Acevedo, Ruiz-Fons, & Vicente, 2006), raising new questions about disease pathogenesis and epidemiology. The increasing role of wildlife in the emergence of livestock diseases is due to multiple changes occurring within wildlife and livestock populations, including encroachment on natural habitats, climate change and alteration of population demographics. Most notably, alteration of wildlife population demographics caused by anthropogenic landscape modification or introduction of non-native species can create new interfaces between livestock and wildlife, potentially exacerbating processes that favour pathogen transmission (Gortazar et al., 2015). Importantly, transmission of an infectious agent at the wildlife/livestock interface may occur directly through interspecies contact, or indirectly through shared space or vectors (Gortazar et al., 2006; Miller, Farnsworth, & Malmberg, 2013).

Australian livestock species are infected with multiple parasitic pathogens of economic relevance, such as *Fasciola hepatica*('liver fluke'), *Echinococcus granulosus* ('hydatids'),*Theileria orientalis* , *Babesia bigemina* , *Babesia bovis* and *Anaplasma marginale* (Bock, deVos, & Molloy, 2006; Jenkins, 2018; Thompson, 2018). Further, infection with *Neospora caninum* has been identified as a major cause of abortion in cattle and

investigations in Queensland have estimated a prevalence of around 20% (Reichel, 2000). Other pathogenic genera such as *Trypanosoma* ,*Sarcocystis* and *Toxoplasma* have also been detected in Australian wildlife species (Munday & Mason, 1980; Pan et al., 2012; Thompson, Godfrey, & Thompson, 2014). These pathogens share a large diversity and distribution of their intermediate hosts and are prevalent in domestic animals, thus they constitute a biosecurity concern for livestock industries (Spratt & Beveridge, 2018).

Australia's livestock export industry was valued at approximately AU$1,780 million for the 2015–16 financial year (Australian Government Department of Agriculture, 2020b). Although Australia is currently free from some of the world's most important livestock diseases such as foot-and-mouth disease and avian influenza H5N1, endemic infectious agents impact on livestock industries. For instance, the economic losses produced by *Neospora caninum* in Australian cattle were estimated at AU$85 million and AU$25 million per annum for the dairy and the beef cattle industries, respectively (Reichel, 2000). Moreover, exotic diseases constitute a major threat to Australia's livestock industry and a severe outbreak would considerably impact Australia's production and access to export markets (Australian Government Department of Agriculture, 2020a).

Among Australian wildlife capable of carrying pathogens transmissible to livestock, deer are of substantial concern as they commonly feed on pasture and crops in agricultural landscapes, achieve high local population densities, are highly mobile and are susceptible to a wide range of viral, bacterial and parasitic infections that may affect other ungulates (Cripps, Pacioni, Scroggie, Woolnough, & Ramsey, 2019). Deer were introduced in Australia as game animals in the mid-19$^{\text{th}}$ century and have successfully adapted to the climate and environmental conditions. In addition to the initial intentional releases, there are records of numerous animals establishing wild populations after escaping from deer farms (Davis et al., 2016). Currently, six non-native deer species have established viable wild populations in most Australian habitats (excluding the arid interior and north-west), and most of these species are expanding their distributions and abundances (Davis et al., 2016; Forsyth, Stamation, & Woodford, 2016).

Numerous pathogens have been detected in various deer species worldwide, including protozoan parasites with epidemiological relevance to humans and domestic animals. For example, serological evidence of*Neospora* and *Toxoplasma* exposure has been detected in fallow (*Dama dama*) and red deer (*Cervus elaphus*) in Poland (Bien, Moskwa, Bogdaszewski, & Cabaj, 2012), Italy (Rocchigiani et al., 2016) and Mexico (De La Torre et al., 2017). Evidence of piroplasm infection (*Babesia* and *Theileria*) was reported in the same two deer species in Europe and China (Garcia-Sanmartin et al., 2007; Hornok et al., 2017; Li et al., 2014; Tampieri et al., 2008; Zanet et al., 2014). Also, *Plasmodium* parasites have been identified in farmed North American white-tailed deer (*Odocoileus virginianus*) (Guggisberg, Sayler, Wisely, & John, 2018) and South American pampas deer (*Ozotoceros bezoarticus*) (Asada et al., 2018).

Limited information is available regarding the overall infection status of Australian wild deer populations. In particular, serological evidence is restricted to parasitic helminths, *Leptospira* and some endemic livestock viruses in red deer (*Cervus elaphus*) from Queensland (McKenzie et al., 1985) and rusa deer (*Rusa timorensis*) from New South Wales (Moriarty, 2004). Importantly, the prevalence of blood-borne parasites known to infect deer overseas has not been investigated in Australian wild deer populations. Therefore, the role that wild deer might play in the spread of these diseases to livestock remains unclear, and the transmission of such pathogens to livestock has yet to be demonstrated in Australia. Addressing this knowledge gap is critical to anticipating how such pathogens might be transmitted to other animals (including livestock and humans), and how these diseases may be controlled. To this end, a molecular survey of parasitic genera previously detected in Australian livestock (*Theileria* ,*Babesia* and *Neospora*), and in deer and livestock overseas (*Trypanosoma* , *Plasmodium* , *Sarcocystis* and*Toxoplasma*), was performed in a wide range of deer blood samples, representing four deer species, collected during 2018 and 2019 across four Australian states and territories.

Materials and methods

Deer blood sample collection

Blood samples were collected by recreational hunters and professional culling staff from deer populations

across eastern Australia (Figure 1). In most cases the geographic location of collection sites was determined based on its proximity with livestock dense areas. Samples were mostly collected during field operations in cold weather months, when most culling operations occur in Australia. Blood was drawn from the jugular vein, the heart or the thoracic cavity and collected with and without anticoagulant (EDTA). Collection tubes (Becton Dickinson, USA) were inverted to mix and prevent clotting, immediately kept under refrigerated conditions and transported to the laboratory. Immediately upon reception, samples were centrifuged for 10 min at 2,000 g. Aliquots of plasma and blood pellet were stored at -80°C and -20°C, respectively.

Genomic DNA extraction from blood using a classical phenol-chloroform method

A small subset of blood samples was randomly selected, and genomic DNA was extracted using a classical phenol-chloroform extraction, a rapid and inexpensive liquid-liquid extraction technique (Chacon-Cortes & Griffiths, 2014). Briefly, 200 µl of blood was lysed with 0.15% saponin at 4°C for 10 minutes and centrifuged at 2,800 g for 10 minutes. The pellet was washed with PBS, resuspended in 700 µl of Lysis buffer (10 mM Tris pH 8, 1 mM EDTA pH 8, 0.4 M NaCl and 1% SDS) and incubated at 37°C for 1 hour. A minimum of two phenol-chloroform extractions were performed by adding 700 µl of phenol-chloroform-isoamyl alcohol mixture (cat#: 77617, Sigma-Aldrich, USA) until the aqueous phase was clear. The DNA present in the aqueous phase was ethanol-precipitated and centrifuged at 9,000 g for 3 minutes at 4°C. The supernatant was discarded, and the pellet washed with 70% ethanol. The DNA pellet was resuspended in TE buffer pH 8 (1 M Tris pH 8 and 0.5 M EDTA pH 8). The concentration and purity of the extracted genomic DNA samples were measured with an IMPLEN Nanophotometer (IMPLEN, Germany).

Genomic DNA extraction from blood using a bead-based automated kit

Extraction of genomic DNA from all blood pellet samples with the bead-based automated kit MagMAXCORE Nucleic Acid Purification Kit (ThermoFisher Scientific, Applied Biosystems, USA) was performed according to the manufacturer's recommendations. Briefly, 200 µL of sample was mixed with a bead/Proteinase K mix and a lysis/binding solution. The mixture was processed using the KingFisher Duo Prime Purification System (ThermoFisher Scientific, USA). Genomic material was eluted in 90 µl of Elution buffer and stored at -20°C until required. The concentration and purity of samples were measured with an IMPLEN Nanophotometer (IMPLEN, Germany).

Molecular detection of parasites by PCR

To generate a positive control for the PCR analysis, genomic DNA extracted from *in vitro* cultures of various parasites and kindly provided by collaborators was used: *Trypanosoma brucei* ,*Plasmodium falciparum* , *Neospora caninum* , *Sarcocystis gigantea* , *Toxoplasma gondii* , *Babesia bovis* and*Theileria orientalis* . The negative control consisted of the PCR mix with nuclease-free water added instead of gDNA. PCR primers targeting a conserved region of the 18S subunit of the ribosomal RNA gene (18S rRNA), or the cytochrome b gene in the case of*Plasmodium* , were obtained from the literature for the seven parasite genera selected in this study (Table 1 and references therein).

PCR amplification was performed in a 25 µl reaction mixture containing 1x Green GoTaq Flexi buffer, 2 mM $MgCl_2$, 10 mM dNTPs, 0.2 µM of both forward and reverse primers (Table 1), 0.625 units of GoTaq G2 DNA polymerase (Promega, USA), and 1 µl of total genomic DNA template. The PCR program consisted of an initial denaturation step at 95ºC for 2 minutes, followed by 35 cycles of denaturation at 95ºC for 45 s, annealing at 55–60ºC for 45 s and extension at 72ºC for 45–90 s, with a final extension of 5 min at 72ºC (see Table 1). Amplification occurred in a T100 thermal cycler (BioRad, USA). Amplification products were visualised by gel electrophoresis, using a 2% agarose gel, RedSafe (iNtRON Biotechnology, Korea), and a high-resolution imaging system (ChemiDoc MP Imaging System, Bio-Rad, USA).

Parasite prevalence and statistical analysis

Mean comparisons were made with t -tests for two independent means, with $\alpha = 0.05$. The maximum possible prevalence of infection, interpreted as the maximum prevalence (for each pathogen) that could be present in a population but not observed (i.e. with all tested samples negative), was calculated with three

confidence levels (90, 95 and 99%) with an unknown population size using WinEpi 2.0 (Thrusfield, Ortega, de Blas, Noordhuizen, & Frankena, 2001).

Results

Host information and statistics

During the sampling period, 243 wild deer blood samples were collected from four wild deer species (fallow, rusa, sambar (*Rusa unicolor*) and chital (*Axis axis*) deer) across eastern states and territories of mainland Australia (Figure 1). Most samples (69%) were collected in June – October (i.e. winter and spring). Among the four deer species included in this study, two accounted for more than 83% of the samples: fallow deer (57%), and rusa deer (26%). Of note, 69% of the specimens were collected in the state of New South Wales (Figure 1). Similar numbers of females (n = 120) and males (n = 116) were sampled, with no sex information available for seven animals. Individuals were classified in three age categories based on morphological characteristics including body size, tooth wear, and antler growth: fawn (< 1 year), yearling (1 to < 2 years) and adult ([?] 2 years). Most samples were from adults (n = 149), followed by yearlings (n = 74) and fawns (n = 13), with age information unavailable for seven animals.

Qualitative analysis of the gDNA samples

Considering the high volume of blood samples processed in this study, we undertook a small-scale comparison to identify the most time-cost efficient DNA extraction method that would provide optimal DNA quality. Ten blood samples were randomly selected, and genomic DNA was extracted using a bead-based method (MagMAXCORE Nucleic Acid Purification Kit) and a classical phenol-chloroform extraction method. Sample quality (i.e. concentration and purity) was assessed by spectrophotometry.

Analysis of the gDNA samples extracted with the phenol-chloroform method determined a mean DNA concentration of 215.3 ng/µl (SD = 35.2, range = 156 – 286), a A260/A230 ratio of 1.7 (SD = 0.098, range = 1.49 – 1.80) and a A260/A280 ratio of 1.45 (SD = 0.02, range = 1.42 – 1.48). On the other hand, the mean DNA concentration obtained with the MagMAXCORE extraction kit was lower (p = 0.0007) (mean 80.4 ng/µl, SD = 81.2, range = 28 – 283), although, these samples presented similar (p = 0.4) A260/A230 ratio (mean 1.7, SD = 0.04, range = 1.64 – 1.76) but a higher (p <0.0001) A260/A280 ratio with a mean of 1.9 (SD = 0.08, range = 1.77 – 2.00). Therefore, the DNA purity with MagMAX extraction method was deemed more consistent and superior when compared to the phenol-chloroform method. Subsequently, MagMAXCORE was used to extract genomic DNA from all the 243 blood samples included in this study.

Pathogen PCR amplification analysis

Conserved primers identified in the literature (see Table 1) were used to amplify the 18S subunit of the ribosomal RNA gene (18S rRNA) of *Trypanosoma brucei* ,*Neospora caninum* , *Sarcocystis gigantea* , *Toxoplasma gondii* , *Babesia bovis* and *Theileria orientalis* (Franco, Romero, Ferrari, Schnittger, & Florin-Christensen, 2018; Thompson et al., 2013; Yang et al., 2014). In the case of *Plasmodium falciparum* , cytochrome b primers (Schaer et al., 2013) were used. Purified genomic DNA of these seven pathogens was obtained from *in vitro* cultures and used to validate the 18S rRNA/cytochrome b primers and PCR amplification conditions. In this study, PCR conditions were slightly modified as indicated in Table 1. PCR amplification of the positive controls resulted in amplicons of the expected size for the seven parasites investigated (Figure 2).

The 243 genomic DNA samples were subject to PCR amplification with the four primer pairs and PCR conditions indicated in Table 1. Positive controls shown in Figure 2 were used in each PCR round. Overall, the PCR analysis did not amplify the 18S rRNA or the cytochrome b gene of the seven selected parasites in any of the 243 samples, while the same genes were successfully amplified in the positive controls within the same experiment. Despite our best efforts to obtain larger numbers of whole blood samples from Queensland (n = 4), we could only access additional serum samples of chital deer from this state (n = 105). Genomic DNA was extracted from a subset of these serum samples (n = 50) and the PCR screen for the seven parasite

genera was performed (same conditions as indicated in Table 1). No amplicons were obtained for any of the 50 serum samples and these are not discussed further.

Parasite prevalence and confidence intervals

The maximum possible prevalence of infection represents the maximum prevalence (for each parasite) that can be estimated considering the total number of samples analysed for each deer species. The maximum possible prevalence of infection ranged from 1.7% to 11.4%, except for chital deer where it ranged from 43.8% to 68.4% (Table 2).

Discussion

This study assessed, for the first time, the prevalence of seven parasite genera in four different wild deer species across Australia. A total of 243 blood samples were analysed by PCR and the presence of *Trypanosoma* , *Plasmodium* , *Neospora* ,*Sarcocystis* , *Toxoplasma* , *Babesia* and *Theileria* was not detected. These findings provide a wide perspective of the current disease status of the four deer species investigated (rusa, sambar, chital and fallow deer), which is important information given that deer coexist and closely interact with humans, domestic animals and other wildlife species.

Although the limited knowledge of parasitic infections in Australian wild deer populations is restricted to helminths (McKenzie et al., 1985; Moriarty, 2004), reports from other countries (Cripps et al., 2019) indicate that the deer species tested in our study are susceptible to the pathogens screened in this survey. Interestingly, experimental infection of white-tailed deer with *Babesia bovis* (Ueti, Olafson, Freeman, Johnson, & Scoles, 2015), a pathogenic parasite in cattle and endemic in Australia (Bock et al., 2006), was reported unsuccessful. This finding raises the question about the epidemiological role of specific wild deer species in the maintenance of pathogens in livestock populations.

Theileria , *Babesia* and *Neospora* are endemic parasites in livestock in the geographical areas covered in our study and with particularly high prevalence in Queensland (Bock et al., 2006; Reichel, 2000). Babesiosis is a well-documented disease of cattle and endemic areas have been reported in northern Australia (Queensland, Western Australia and Northern Territory) (Bock et al., 2006). Bovine theileriosis currently occurs throughout coastal New South Wales and Victoria, Queensland and some isolated parts of South Australia and Western Australia (Jenkins, 2018). The prevalence of *Neospora caninum* , identified as a major cause of infectious abortion in Australian cattle, was estimated to be around 20% in one study (Reichel, 2000). It is therefore conceivable that wild deer might carry some of these parasites. However, the low number of samples collected in Queensland in this study, 1.6% (4/243), decreases the probability of detecting infected animals despite the high prevalence of the parasite in livestock species in this region.

Reports of Australian wildlife infections with *Plasmodium*parasites are limited and restricted to Leadbeater's possum (*Gymnobelideus leadbeateri*) (Scheelings, McLaren, Tatarczuch, & Slocombe, 2016), birds (Grim, McCutchan, Sullivan, & Cranfield, 2008; Spratt & Beveridge, 2018) and reptiles (Spratt & Beveridge, 2018; Telford, 1979). In contrast, *Trypanosoma* , *Sarcocystis* and *Toxoplasma* have been widely identified in Australian wildlife (Spratt & Beveridge, 2018). For instance, *Trypanosoma* has been detected in indigenous mammalian fauna living in the same Australian regions targeted in this study. In total, eight native species of *Trypanosoma* have been described and exotic trypanosomes have been identified from introduced mammals (Thompson et al., 2014). In Australia, wild deer coexist with livestock but are also sympatric with other wildlife species. Thus, considering the diversity and distribution of intermediate hosts for pathogens such as trypanosomes, wild deer constitute an obvious biosecurity concern. Our study has found no evidence of trypanosome infections in the blood of Australian wild deer, nor of the other six parasites tested, which may be dependent on several factors, including season of the sampling, low parasitaemia at the time of blood collection and fluctuation of parasitaemia during the parasite's life cycle.

One of the limitations of this study is that samples analysed were mostly collected during the Australian winter and spring seasons, with 69% of the samples being collected between June and October. Most of the sampling occurred during cold weather months due to logistical reasons outside of our control. Cold

weather conditions negatively influence the transmission rates of vector-borne diseases (Caminade, McIntyre, & Jones, 2019); therefore, sampling season may impact the chances of detecting infected animals.

Extremely low parasite loads have been previously reported in wild deer. *Plasmodium odocoilei* was estimated to infect ˜1/65,000 red blood cells of white-tailed deer (Martinsen et al., 2016; Templeton, Asada, et al., 2016; Templeton, Martinsen, Kaewthamasorn, & Kaneko, 2016), and molecular tests have previously determined *Plasmodium* parasitaemia levels (percentage of infected red blood cells) in cervids to be as low as 0.003% (Martinsen et al., 2016; Templeton, Asada, et al., 2016). This low blood parasite burden observed in adult individuals has led to the hypothesis that blood-stage ungulate malaria is best characterised as a chronic, occult infection without major health consequences (Templeton, Martinsen, et al., 2016). Further, it is important to highlight that molecular screening such as conducted in this study can only identify an active infection (i.e. animals that have recovered from infection will not be identified as they would be via serology). A variety of PCR-based assays are now widely used for detection of parasite DNA in blood (Garcia-Sanmartin et al., 2007; Li et al., 2014; Remesar et al., 2019; Yang et al., 2014). In our study, we minimised the effects of PCR inhibitors and the presence of false negatives by obtaining high-quality DNA for each sample, however molecular assays are not infallible.

The opportunistic nature of our study allowed us to collect many samples, but each sample was collected at a single time point (i.e. no serial sampling of animals). Hence, the parasite life cycle at the time of blood collection may have had a direct impact on the negative findings of our study.

Despite the lack of evidence of current infection in the 243 blood samples analysed in this study, the maximum possible prevalence was calculated for each deer species (Table 2) and found to range between 3.5 and 11.4% for fallow, rusa and sambar deer with a 99% confidence level. Since the maximum possible prevalence is directly influenced by the number of samples screened, the maximum prevalence obtained for chital deer (n = 4) greatly exceeds the values obtained for the other three deer species given the limited number of animals sampled. We acknowledge our very limited sample size and recommend further studies to expand on our initial screening of chital deer blood parasites in Queensland.

Importantly, the impact of climate change might be considered as a factor affecting the spread of wildlife diseases in new areas. Modelling suggests that, by 2100, the average global temperature will have risen by 1.0–3.5^{o}C (Githeko, Lindsay, Confalonieri, & Patz, 2000). Climate change will strongly affect the distribution, abundance and transmission rates, the intensity and temporal pattern of vector activity; and the survival and reproduction of pathogens within vectors. This will increase the likelihood of vector-borne diseases in new areas (Duncan, Backus, Lynn, Powers, & Salman, 2008; Guberti, Stancampiano, & Ferrari, 2014). The impact of climate change is evident in Europe with *Ixodes* ticks, vectors of several pathogens including *Babesia* and *Anaplasma* , where, over the past decade, an expansion in vectors' geographical range (spread to northern areas) and increase in activity were observed as consequence of milder winters and prolonged spring and autumn seasons, combined with increased vegetative cover and the spread of animals carrying ticks into newly suitable regions (Caminade et al., 2019). Although all the samples screened in this study were negative for the parasites tested, the presence of suitable vectors and previous evidence of infection in domestic and wild animals (Bock et al., 2006; Spratt & Beveridge, 2018; Thompson et al., 2014) indicates that climate change could transform current pathogen-free regions into 'new habitats' for vectors and pathogens.

In the context of animal health, wildlife disease surveillance is an important tool to obtain information of morbidity and mortality, changes in patterns of disease occurrence over time, and early detection of disease outbreaks, including those linked to emerging diseases (Duncan et al., 2008; Grogan et al., 2014). For example, surveillance programs in Europe resulted in detection of a new disease in rabbits, the European brown hare syndrome caused by a calicivirus (Artois et al., 2009). However, detection of a new disease depends on its prevalence, patterns of transmission and disease-induced mortality. Therefore, sampling effort is crucial. Our sampling methodology involved recreational hunters and staff in deer control programs, who provided samples and data, but obtaining information on the health status of deer at the time of sampling was challenging. Considering the likelihood of chronic and low burden parasitic infections of deer, a key factor that would increase the efficacy and efficiency of wildlife disease surveillance is a clearer definition of

the term 'suspect case'. This description could be available and shared with the wide range of stakeholders including hunters and wildlife rangers who could be involved in retrieving important information during their traditional activities (Grogan et al., 2014; Guberti et al., 2014). In Australia, recreational hunting is an economically important activity, contributing AU$335 to the economy, with deer among the primary species hunted (Australian Government Department of Health, 2019). In Victoria alone there were over 36,000 licensed deer hunters that harvested ˜100,000 deer in 2018 (Victoria State Government, 2019). This large hunter population, if utilised to enable wildlife disease surveillance (Ryser-Degiorgis, 2013), represents an opportunity to implement a passive surveillance program to detect and identify endemic and emerging infections in new areas.

The focus of this study was on parasites, but the approach taken could be readily expanded to consider bacterial and viral pathogens. Our data suggest that it is unlikely that a large proportion of Australian deer are involved in maintaining the life cycle of *Trypanosoma* ,*Plasmodium* , *Neospora* , *Sarcocystis* ,*Toxoplasma* , *Babesia* and *Theileria* , as our maximum possible prevalence of infection are lower than those reported in Italy (Zanet et al., 2014) and Canada (Milnes et al., 2019). Importantly, this survey represents the first molecular study of its type in Australian deer and provides important baseline information about the disease status of wild deer in eastern Australia. Despite our best efforts, we could not conduct extensive deer sampling in warmer months or in tropical areas. Considering the additional limitations of our study discussed above, further studies combining serology assays and high-throughput sequencing are desirable. This would enhance parasite detection, and ultimately characterise the epidemiology of such pathogens in Australian wild deer populations. Assessment of the infection status of invasive species such as deer is necessary for future planning and successful implementation of disease eradication programs in livestock (Gortazar et al., 2015).

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Conflict of Interest Statement

No potential conflict of interest was reported by the authors.

Data availability statement

The data that supports the findings of this study are available in the various sections of this article. If needed, further information is available from the corresponding author upon request.

Ethical statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as samples for this study were collected from carcasses. Relevant permits for the culling programs that facilitated the sample collections are held by the organizations that organized and conducted the culling.

References

Artois, M., Bengis, R., Delahay, R. J., Duchêne, M.-J., Duff, J. P., Ferroglio, E., . . . Smith, G. C. (2009). Wildlife Disease Surveillance and Monitoring. In R. J. Delahay, G. C. Smith, & M. R. Hutchings (Eds.), *Management of Disease in Wild Mammals* (pp. 187-213). Tokyo: Springer Japan.

Asada, M., Takeda, M., Tomas, W. M., Pellegrin, A., de Oliveira, C. H. S., Barbosa, J. D., . . . Kaneko, O. (2018). Close relationship of Plasmodium sequences detected from South American pampas deer (Ozotoceros bezoarticus) to Plasmodium spp. in North American white-tailed deer. *Int J Parasitol Parasites Wildl, 7* (1), 44-47. doi:10.1016/j.ijppaw.2018.01.001

Australian Government Department of Agriculture, Water and the Environment (2020a). Animal pests and diseases. Retrieved from https://www.agriculture.gov.au/pests-diseases-weeds/animal

Australian Government Department of Agriculture, Water and the Environment (2020b). Livestock. Retrieved from https://www.agriculture.gov.au/export/controlled-goods/live-animals/livestock

Bien, J., Moskwa, B., Bogdaszewski, M., & Cabaj, W. (2012). Detection of specific antibodies anti-Neospora caninum in the fallow deer (Dama dama). *Res Vet Sci, 92* (1), 96-98. doi:10.1016/j.rvsc.2010.10.014

Bock, R. E., deVos, A. J., & Molloy, J. B. (2006). Tick-borne diseases. In *Australian and New Zealand Standard Diagnostic Procedures* . Australia

Caminade, C., McIntyre, K. M., & Jones, A. E. (2019). Impact of recent and future climate change on vector-borne diseases. *Ann N Y Acad Sci, 1436* (1), 157-173. doi:10.1111/nyas.13950

Chacon-Cortes, D., & Griffiths, L. R. (2014). Methods for extracting genomic DNA from whole blood samples: current perspectives.*Journal of Biorepository Science for Applied Medicine* (2), 1-9. doi:10.2147/BSAM.S46573

Cripps, J. K., Pacioni, C., Scroggie, M. P., Woolnough, A. P., & Ramsey, D. S. L. (2019). Introduced deer and their potential role in disease transmission to livestock in Australia. *Mammal Review, 49* (1), 60-77. doi:10.1111/mam.12142

Davis, N. E., Bennett, A., Forsyth, D. M., Bowman, D. M. J. S., Lefroy, E. C., Wood, S. W., . . . Johnson, C. N. (2016). A systematic review of the impacts and management of introduced deer (family Cervidae) in Australia. *Wildlife Research, 43* (6), 515-532. doi:10.1071/Wr16148

De La Torre, J. R., Bautista-Pina, C., Alfonso Ortega, S. J., Cantu-Covarruvias, A., Genoveva Alvarez-Ojeda, M., Romero-Salas, D., . . . Bryant, F. C. (2017). Neospora caninum in Axis Deer (Axis axis) and Fallow Deer (Dama dama) in Northern Mexico. *J Wildl Dis, 53* (1), 186-187. doi:10.7589/2016-05-095

Duncan, C., Backus, L., Lynn, T., Powers, B., & Salman, M. (2008). Passive, opportunistic wildlife disease surveillance in the Rocky Mountain Region, USA. *Transbound Emerg Dis, 55* (7), 308-314. doi:10.1111/j.1865-1682.2008.01039.x

Forsyth D.M., Stamation K., & Woodford L. (2016). Distributions of Fallow Deer, Red Deer, Hog Deer and Chital Deer in Victoria. Retrieved from https://www.parliament.vic.gov.au/images/stories/committees/enrc/Invasive_Animals_on_Crown_-land/210Q._2016.09.13_Attachment_17_-_Fallow_Deer_Red_Deer_Hog_

Deer_and_Chital_Deer_in_Victoria.pdf

Franco, C. D., Romero, S., Ferrari, A., Schnittger, L., & Florin-Christensen, M. (2018). Detection of Sarcocystis aucheniae in blood of llama using a duplex semi-nested PCR assay and its association with cyst infestation. *Heliyon, 4* (11). doi:ARTN e0092810.1016/j.heliyon.2018.e00928

Garcia-Sanmartin, J., Aurtenetxe, O., Barral, M., Marco, I., Lavin, S., Garcia-Perez, A. L., & Hurtado, A. (2007). Molecular detection and characterization of piroplasms infecting cervids and chamois in Northern Spain. *Parasitology, 134* (Pt 3), 391-398. doi:10.1017/S0031182006001569

Githeko, A. K., Lindsay, S. W., Confalonieri, U. E., & Patz, J. A. (2000). Climate change and vector-borne diseases: a regional analysis.*Bull World Health Organ, 78* (9), 1136-1147.

Gortazar, C., Acevedo, P., Ruiz-Fons, F., & Vicente, J. (2006). Disease risks and overabundance of game species. *European Journal of Wildlife Research, 52* (2), 81-87. doi:10.1007/s10344-005-0022-2

Gortazar, C., Diez-Delgado, I., Barasona, J. A., Vicente, J., De La Fuente, J., & Boadella, M. (2015). The wild side of disease control at the wildlife-livestock-human interface: a review. *Frontiers in Veterinary Science, 2* . doi:10.3389/fvets.2014.00027

Grim, K. C., McCutchan, T., Sullivan, M., & Cranfield, M. R. (2008). Unidentified Plasmodium species in Australian black swans (Cygnus atratus) hatched and raised in North America. *J Zoo Wildl Med, 39* (2), 216-220. doi:10.1638/2007-0110R.1

Grogan, L. F., Berger, L., Rose, K., Grillo, V., Cashins, S. D., & Skerratt, L. F. (2014). Surveillance for emerging biodiversity diseases of wildlife. *PLoS Pathog, 10* (5), e1004015. doi:10.1371/journal.ppat.1004015

Guberti, V., Stancampiano, L., & Ferrari, N. (2014). Surveillance, monitoring and surveys of wildlife diseases: a public health and conservation approach. *Hystrix-Italian Journal of Mammalogy, 25* (1), 3-8. doi:10.4404/hystrix-25.1-10114

Guggisberg, A. M., Sayler, K. A., Wisely, S. M., & John, A. R. O. (2018). Natural History of Plasmodium odocoilei Malaria Infection in Farmed White-Tailed Deer. *Msphere, 3* (2).

Australian Government Department of Health (2019). Economic and social impacts of recreational hunting and shooting. Retrieved from https://www1.health.gov.au/

internet/main/publishing.nsf/Content/economic-and-social-impacts-of-recreational-hunting-and-shooting

Hornok, S., Sugar, L., Horvath, G., Kovacs, T., Micsutka, A., Gonczi, E., . . . Hofmann-Lehmann, R. (2017). Evidence for host specificity of Theileria capreoli genotypes in cervids. *Parasit Vectors, 10* (1), 473. doi:10.1186/s13071-017-2403-2

Jenkins, C. (2018). Bovine theileriosis in Australia: a decade of disease. *Microbiology Australia, 39* (4), 215-219. doi:10.1071/Ma18067

Li, Y., Chen, Z., Liu, Z., Liu, J., Yang, J., Li, Q., . . . Yin, H. (2014). Molecular identification of Theileria parasites of northwestern Chinese Cervidae. *Parasit Vectors, 7* , 225. doi:10.1186/1756-3305-7-225

Martinsen, E. S., McInerney, N., Brightman, H., Ferebee, K., Walsh, T., McShea, W. J., . . . Fleischer, R. C. (2016). Hidden in plain sight: Cryptic and endemic malaria parasites in North American white-tailed deer (Odocoileus virginianus). *Sci Adv, 2* (2), e1501486. doi:10.1126/sciadv.1501486

McKenzie, R. A., Green, P. E., Thornton, A. M., Chung, Y. S., MacKenzie, A. R., Cybinski, D. H., & St George, T. D. (1985). Diseases of deer in south eastern Queensland. *Aust Vet J, 62* (12), 424. doi:10.1111/j.1751-0813.1985.tb14129.x

Miller, R. S., Farnsworth, M. L., & Malmberg, J. L. (2013). Diseases at the livestock-wildlife interface: status, challenges, and opportunities in the United States. *Preventive Veterinary Medicine, 110* (2), 119-132. doi:10.1016/j.prevetmed.2012.11.021

Milnes, E. L., Thornton, G. L., Delnatte, P., Leveille, A. N., Barta, J. R., Smith, D. A., & Nemeth, N. M. (2019). Molecular Detection of Babesia Odocoilei in Wild, Farmed, and Zoo Cervids in Ontario, Canada.*J Wildl Dis, 55* (2), 335-342. doi:10.7589/2018-06-147

Moriarty, A. (2004). *Ecology and enviromental impact of Javan rusa deer (Cervus timorensis russa) in the Royal National Park.* (PhD PhD thesis), University of Western Sydney, Sydney.

Munday, B. L., & Mason, R. W. (1980). Sarcocystis and related organisms in Australian wildlife: III. Sarcocystis murinotechis sp.n. life cycle in rats (Rattus, Pseudomys and Mastocomys spp.) and tiger snakes (Notechis ater). *J Wildl Dis, 16* (1), 83-88. doi:10.7589/0090-3558-16.1.83

Pan, S., Thompson, R. C., Grigg, M. E., Sundar, N., Smith, A., & Lymbery, A. J. (2012). Western Australian marsupials are multiply infected with genetically diverse strains of Toxoplasma gondii.*PLoS One, 7* (9), e45147. doi:10.1371/journal.pone.0045147

Reichel, M. P. (2000). Neospora caninum infections in Australia and New Zealand. *Aust Vet J, 78* (4), 258-261. doi:10.1111/j.1751-0813.2000.tb11751.x

Remesar, S., Diaz, P., Prieto, A., Markina, F., Cao, J. M. D., Lopez-Lorenzo, G., . . . Morrondo, P. (2019). Prevalence and distribution of Babesia and Theileria species in roe deer from Spain.*International Journal for Parasitology-Parasites and Wildlife, 9* , 195-201. doi:10.1016/j.ijppaw.2019.05.005

Rocchigiani, G., Nardoni, S., D'Ascenzi, C., Nicoloso, S., Picciolli, F., Papini, R. A., & Mancianti, F. (2016). Seroprevalence of Toxoplasma gondii and Neospora caninum in red deer from Central Italy. *Ann Agric Environ Med, 23* (4), 699-701. doi:10.5604/12321966.1226870

Ryser-Degiorgis, M. P. (2013). Wildlife health investigations: needs, challenges and recommendations. *BMC Vet Res, 9* , 223. doi:10.1186/1746-6148-9-223

Schaer, J., Perkins, S. L., Decher, J., Leendertz, F. H., Fahr, J., Weber, N., & Matuschewski, K. (2013). High diversity of West African bat malaria parasites and a tight link with rodent Plasmodium taxa.*Proc Natl Acad Sci U S A, 110* (43), 17415-17419. doi:10.1073/pnas.1311016110

Scheelings, T. F., McLaren, P. J., Tatarczuch, L., & Slocombe, R. F. (2016). Plasmodium infection in a Leadbeater's possum (Gymnobelideus leadbeateri). *Aust Vet J, 94* (8), 299-303. doi:10.1111/avj.12466

Spratt, D. M., & Beveridge, I. (2018). Wildlife parasitology in Australia: past, present and future. *Australian Journal of Zoology, 66* (4), 286-305. doi:10.1071/Zo19017

Tampieri, M. P., Galuppi, R., Bonoli, C., Cancrini, G., Moretti, A., & Pietrobelli, M. (2008). Wild ungulates as Babesia hosts in northern and central Italy. *Vector Borne Zoonotic Dis, 8* (5), 667-674. doi:10.1089/vbz.2008.0001

Telford, S. R., Jr. (1979). A malarial parasite of Australian skinks, Plasmodium mackerrasae sp. n. *J Parasitol, 65* (3), 409-413.

Templeton, T. J., Asada, M., Jiratanh, M., Ishikawa, S. A., Tiawsirisup, S., Sivakumar, T., . . . Kaneko, O. (2016). Ungulate malaria parasites.*Sci Rep, 6* , 23230. doi:10.1038/srep23230

Templeton, T. J., Martinsen, E., Kaewthamasorn, M., & Kaneko, O. (2016). The rediscovery of malaria parasites of ungulates.*Parasitology, 143* (12), 1501-1508. doi:10.1017/S0031182016001141

Thompson, C. K., Botero, A., Wayne, A. F., Godfrey, S. S., Lymbery, A. J., & Thompson, R. C. (2013). Morphological polymorphism of Trypanosoma copemani and description of the genetically diverse T. vegrandis sp. nov. from the critically endangered Australian potoroid, the brush-tailed bettong (Bettongia penicillata (Gray, 1837)). *Parasit Vectors, 6* , 121. doi:10.1186/1756-3305-6-121

Thompson, C. K., Godfrey, S. S., & Thompson, R. C. (2014). Trypanosomes of Australian mammals: A review. *Int J Parasitol Parasites Wildl, 3* (2), 57-66. doi:10.1016/j.ijppaw.2014.02.002

Thompson, R. C. A. (2018). Exotic Parasite Threats to Australia's Biosecurity-Trade, Health, and Conservation. *Trop Med Infect Dis, 3* (3). doi:10.3390/tropicalmed3030076

Thrusfield, M., Ortega, C., de Blas, I., Noordhuizen, J. P., & Frankena, K. (2001). WIN EPISCOPE 2.0: improved epidemiological software for veterinary medicine. *Vet Rec, 148* (18), 567-572. doi:10.1136/vr.148.18.567

Ueti, M. W., Olafson, P. U., Freeman, J. M., Johnson, W. C., & Scoles, G. A. (2015). A Virulent Babesia bovis Strain Failed to Infect White-Tailed Deer (Odocoileus virginianus). *PLoS One, 10* (6), e0131018. doi:10.1371/journal.pone.0131018

Victoria State Government, Game Managemnet Authority (2019). Deer. Retrieved from https://www.gma.vic.gov.au/hunting/deer

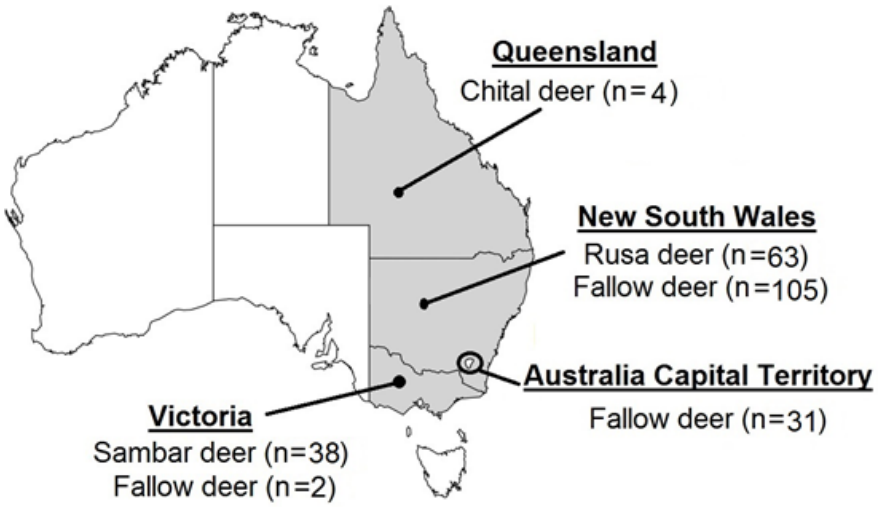
Yang, J. F., Li, Y. Q., Liu, Z. J., Liu, J. L., Guan, G. Q., Chen, Z., . . . Yin, H. (2014). Molecular evidence for piroplasms in wild Reeves' muntjac (Muntiacus reevesi) in China. *Parasitology International, 63* (5), 713-716. doi:10.1016/j.parint.2014.06.002

Zanet, S., Trisciuoglio, A., Bottero, E., de Mera, I. G. F., Gortazar, C., Carpignano, M. G., & Ferroglio, E. (2014). Piroplasmosis in wildlife: Babesia and Theileria affecting free-ranging ungulates and carnivores in the Italian Alps. *Parasites & Vectors, 7* . doi:Artn 7010.1186/1756-3305-7-70

Figure legend

Figure 1 . Map of Australia indicating where sampling was carried out and detailing the number of animals sampled by jurisdiction and the deer species, chital deer (*Axis axis*), rusa deer (*Rusa timorensis*), sambar deer (*Rusa unicolor*) and fallow deer (*Dama dama*). Sample size is reported for whole blood samples only.

Figure 2 . PCR amplification of the 18S rRNA gene from*Babesia bovis* (1409bp), *Neospora caninum* (400bp),*Sarcocystis gigantea* (400bp), *Theileria orientalis*(1409bp), *Toxoplasma gondii* (400bp), *Trypanosoma brucei*(950bp), and cytochrome b gene from *Plasmodium falciparum* (750 bp).



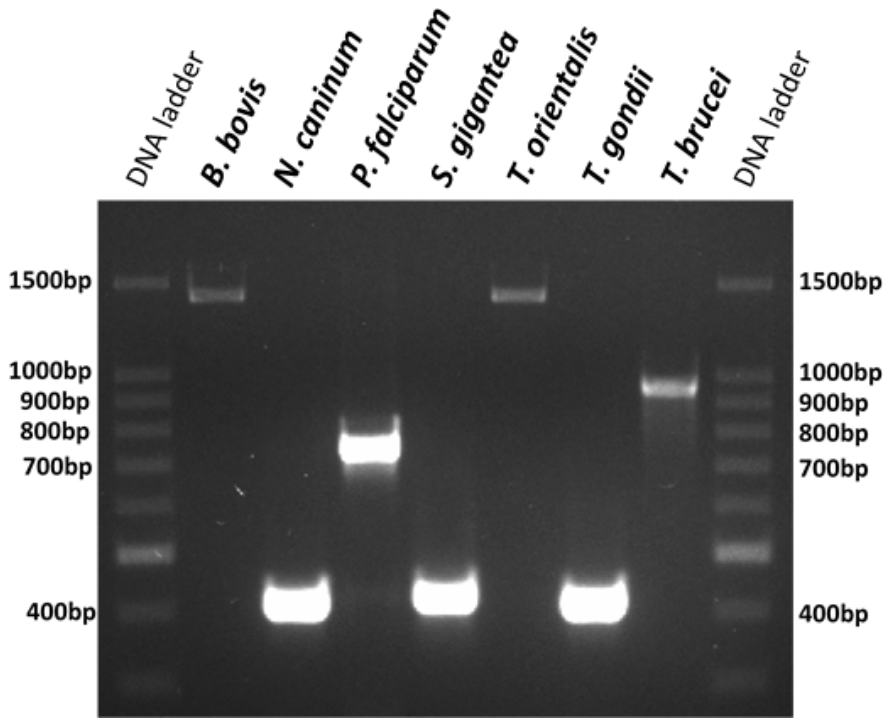


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Table 2.docx available at https://authorea.com/users/321604/articles/450825-screening-of-blood-parasites-in-australian-wild-deer